16 Predictive Biomarkers of Melanoma

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16.1 Diagnostic Biomarkers

16.1.1 S100

The S100 protein family was first identified in glial cells and has since been used as a marker for several tumors, including melanoma [2, 3]. These dimeric calcium sensors play a role in numerous cellular processes, including cell cycle, apoptosis, cell motility, and differentiation [4]. S100 is among the most commonly used IHC markers for melanoma, having first been identified in melanoma in 1980 [3]. The utility of S100 in the diagnosis of melanoma is a function of its high sensitivity, with over 90% of melanoma tumors staining positive for S100 [5, 6]. However, its specificity is low, estimated to be between 70–87% [7–9], given its expression in a number of different tissues.

16.1.2 HMB-45

HMB-45, a monoclonal antibody that recognizes gp100, has been shown to be highly specific for melanoma. Several studies, in fact, have demonstrated 100% specificity for melanoma [10–12]. Its sensitivity, however, ranges from 69–93%, with higher sensitivity observed in primary compared to metastatic melanomas [7]. In addition, it has been shown to be unreliable in the detection of nodal disease [13], suggesting that the most useful application of HMB-45 is in conjunction with other markers.

16.1.3 Melan A

Melan A, also known as MART-1, is a cell surface protein expressed in primary human melanocytes and melanomas recognized by autologous T-cells [14]. It is expressed in melanomas, benign nevi, and normal melanocytes as well as perivascular epithelioid cell tumors (PEComas), clear cell sarcomas, adrenal cortical tumors, and some sex cord stromal tumors. While it has lower sensitivity than S100, it is superior in terms of specificity, with many studies reporting >95% specificity for melanoma versus other malignancies [7, 15]. Melan A has higher sensitivity in primary melanomas (~85–97%) compared to metastatic (57–92%) [6]. Because it is not expressed in the dendritic cells in the lymph

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R. M. Alani, D. Sahni (eds.), *Melanoma in Clinical Practice*,
https://doi.org/10.1007/978-3-030-82639-0_16

nodes, it is superior to S-100 and HMB-45 in detecting microsatellites in sentinel lymph nodes [7, 13]. In addition, it is one of the recommended stains during Mohs micrographic surgery given its high sensitivity in frozen sections [16, 17].

16.1.4 Chondroitin Sulfate Proteoglycan 4 (CSPG4)

Chondroitin sulfate proteoglycan 4 (CSPG4), also known as high molecular weight melanoma-associated antigen or melanoma chondroitin sulfate proteoglycan, is involved in tissue development, cell adhesion and motility, and possibly metastasis [18]. It is expressed in >85% of primary and metastatic melanomas [19, 20]. It has shown superiority to Melan A, S-100, and HMB-45 in staining metastatic lesions, with >90% sensitivity [21]. Moreover, it is particularly useful for diagnosing desmoplastic melanoma, showing greater sensitivity compared to HMB-45 and Melan A [22].

16.2 Prognostic Biomarkers

16.2.1 Immunohistochemical Markers

16.2.1.1 Mitotic Rate

Mitotic rate, while no longer included in the American Joint Commission on Cancer (AJCC) melanoma staging system, is nonetheless a significant predictor of patient survival. Higher mitotic rate in a primary melanoma correlates with lower survival probability, and is the second most significant predictor of melanoma-specific survival after tumor thickness [23].

16.2.1.2 Ki-67

Ki-67 is a commonly used marker of cell proliferation that is expressed during all active stages of the cell cycle (late G_1, S, G_2, and M) [24] and is therefore sometimes used as an alternative to mitotic count [25]. The utility of Ki-67 in determining prognosis in melanoma is somewhat controversial. While Ostmeier et al. reported Ki-67 to be an independent prognostic factor in primary melanomas [26], other studies suggest that the relationship between Ki-67 and poorer clinical outcomes is mediated by other clinicopathologic features, such as ulceration [27, 28]. Additionally, there is conflicting evidence regarding the correlation between Ki-67 and tumor thickness. Moretti et al. found a positive correlation between Ki-67 staining and metastatic activity in melanomas <1.5 mm thick, while there was a negative correlation in primary melanomas >1.5 mm thick [29]. However, other studies have reported an opposite trend, finding the association only in thick melanomas [30–32].

16.2.1.3 Melanoma Cell Adhesion Molecule (MCAM)

Melanoma cell adhesion molecule (MCAM or Mel-CAM), also known as MUC18 or CD146, is a cell adhesion molecule that plays a role in the invasiveness and motility of melanoma. It is highly expressed in both primary and metastatic melanoma [33]. Non-metastatic melanoma cells transfected with MCAM showed increased metastatic potential and tumorigenicity compared to controls [34]. Prospective studies investigating the relationship between MCAM expression and patient outcomes found that increase in MCAM staining intensity was associated with decreased survival [35]. Furthermore, MCAM expression was independently predictive of survival and development of metastases in patients meeting criteria for sentinel lymph node biopsy (SLNB), suggesting that MCAM expression may have utility in stratifying SNLB based on risk [36].

16.2.1.4 Multiple Marker Arrays

Although the biomarkers discussed above have each shown diagnostic and prognostic value, they are all limited by either their sensitivity or specificity. More recently, Alonso et al. used a tissue microarray (TMA) study to analyze 165 malignant melanoma tumors. They identified a predictor model with four antibodies (Ki67, $p16^{INK4a}$, $p21^{CIP1}$, and Bcl-6) that was associated with shorter overall survival (OS) in patients with ver-

tical growth phase melanoma [37]. Kashani-Sabet and colleagues have developed two multi-marker assays for use in melanoma diagnosis and prognosis. The first, a five marker diagnostic assay consisting of ARPC2, FN1, RGS1, SPP1, and WNT2, was 95% specific and 91% sensitive in distinguishing melanoma from benign and dysplastic nevi [38]. The second study identified an array of three biomarkers (NCOA3, SPP1, and RGS1) that was found to be an independent prognostic predictor of disease-specific survival [39]. Gould-Rothberg et al. used the Automated Quantitative Analysis (AQUA) method for immunofluorescence staining and identified five key markers (ATF2, $p21^{WAF1}$, $p16^{INK4A}$, β-catenin, and fibronectin) that distinguished high- and low-risk groups for melanoma-specific mortality [40]. A more recent study included seven biomarkers (Bax, Bcl-X, PTEN, COX-2, loss of β-catenin, loss of MTAP, and presence of CD-20 positive B-lymphocytes) in their model, which was an independent negative predictor for OS and recurrence-free survival (RFS) [41]. While these IHC panels are likely to be more useful at determining prognosis in melanoma than individual biomarkers, their clinical utility remains to be determined.

16.2.2 Genetic Biomarkers

16.2.2.1 KIT

KIT is a receptor tyrosine kinase (RTK) that plays a role in the development of numerous cell lineages including melanocytes, mast cells, and hematopoietic progenitor cells [42]. Amplifications and activating mutations of KIT have been observed at increased frequency in melanomas of mucosal, acral, and chronically sun damaged skin [43]. While early studies treating melanoma patients with imatinib showed limited clinical efficacy and significant toxicity; these studies did not select for patients with KIT mutations or amplifications [1, 44–46]. More recent studies in melanoma patients harboring activating KIT alterations have demonstrated significant efficacy of RTK inhibitors [47–51].

16.2.2.2 Cdkn2a/b

While UV radiation is a known environmental risk factor for melanoma, large pedigrees of familial melanomas have allowed for the identification of heritable genetic mutations associated with a predisposition to melanoma [52]. Two genes associated with a predisposition to melanoma, CDKN2A and CDKN2B, are located in the INK4 locus on chromosome 9p21 and encode tumor suppressor proteins [53]. Germline CDKN2A mutations have been observed in an estimated 20% of tested melanoma families [52, 54–56]. CDKN2A encodes p16 and $p14^{ARF}$. The p16 protein inhibits CDK4 and CDK6, thereby preventing the formation of CDK/Cyclin D complexes that phosphorylate and activate the retinoblastoma protein. Loss of p16 results in uninhibited cell cycle progression and contributes to tumorigenesis [56, 57]. The $p14^{ARF}$ protein acts through the p53 pathway to allow cell cycle arrest and apoptosis [58, 59]. Partial or complete deletion of the INK4 gene cluster has been observed in most melanoma cell lines and in almost half of melanoma metastases [60–62]. Conway et al. found that reduced gene dosage of the regions of 9p21 encoding CDKN2A, CDKN2B, and P14ARF was associated with increased tumor thickness, mitotic rate, and ulceration [59]. Similarly, Grafström et al. reported that monoallelic or biallelic deletions in the INK4 region were associated with reduced median survival [62].

16.2.2.3 Expression Profiling

Gene expression profiling (GEP), which involves measuring the expression of a panel of genes using mRNA, has been used to predict prognosis and response to therapy for a number of different cancers [63]. While there are commercially available GEP tests marketed as being able to classify cutaneous melanoma based on the risk of metastasis, it remains unclear whether the use of GEP tests provide any additional prognostic information in comparison with or in addition to known clinicopathologic factors (patient age, sex, tumor location, thickness, ulceration, SLNB status, lymphovascular invasion, microsatellites, and

mitotic rate), according to the 2020 National Comprehensive Cancer Network (NCCN) guidelines [64]. Winnepenninckx et al. performed the first study linking gene expression profiling of melanoma to clinical outcome and identified 254 genes that were associated with distant metastasis-free survival of patients with primary melanoma [65]. In stage III melanoma, a set of 21 genes was identified that accurately predicted clinical outcome in 85–90% of patients [63]. Jönsson et al. performed hierarchical clustering of 3000 genes from stage IV melanomas and found four tumor subtypes characterized by expression of immune response, pigmentation, proliferation, or stromal composition [66]. They observed a different prognosis between subtypes, with the proliferative subtype associated with the worst survival [66]. Several other studies have identified gene profiles in subsets of melanoma patients that predict clinical outcomes [67–69]. In 2015, Gerami et al. identified a 28-gene signature that classifies tumors as either low risk (class 1) or high risk (class 2) of metastasis [70]. A diagnostic test comprised of these 28 genes, along with 3 control genes, has since been developed, called DecisionDx-Melanoma. Its prognostic utility in predicting recurrence and metastasis has been validated in three prospective studies [71, 72], and the test is now covered by Medicare and Medicaid for patients over 65 years old with T1a, T1b, and T2 tumors [73]; however, its ability to provide clinically actionable prognostic information remains to be determined.

16.2.2.4 MicroRNA (miRNA)

miRNAs are short non-coding RNAs that act post-transcriptionally to modify gene expression and have been shown to be differentially expressed in melanoma compared to healthy controls [74]. Circulating miRNA expression has been found to have the potential to improve the diagnosis, prognosis, and monitoring of response to treatment in melanoma patients [75, 76]. While several studies have found single miRNA expression (miR-16, miR-206, miR-210, miR-15b, miR-205, miR-29c, miR-221, miR-21) to correlate with melanoma disease stage, survival, tumor burden, and recurrence [77–85]; others have focused on developing miRNA expression panels to improve diagnostic and prognostic accuracy. A miRNA array from 59 melanoma metastases identified a signature of 18 miRNAs whose overexpression was significantly associated with survival [86]. Stark et al. developed an miRNA panel of seven miRNAs that was able to detect melanoma with high sensitivity (93%) and specificity (82%) and was reported to be superior to LDH and S100B for melanoma progression, recurrence, and survival [87]. Analysis of 355 miRNAs in the sera of 80 melanoma patients at primary diagnosis revealed a signature of 5 miRNAs classifying melanoma patients into high and low recurrence risk groups and 4 miRNAs that varied dynamically with tumor burden [88], while analysis of serum levels of 12 miRNAs from 283 melanoma patients at diagnosis found a panel of four miRNAs to be predictive of RFS, OS, and recurrence in combination with stage [89]. To date, no single miRNA or miRNA panel has been proven to be an actionable clinical biomarker.

16.2.2.5 Circulating Tumor DNA (ctDNA)

Levels of circulating tumor DNA (ctDNA) in cancer patients are associated with tumor burden, cell turnover, and location of metastasis [90]. BRAF and NRAS mutations occur in approximately 50–70% and 20% of melanomas, respectively [91, 92], and may be detected in peripheral blood of melanoma patients arising from necrotic or apoptotic circulating tumor cells. In melanoma patients with early stage disease, ctDNA levels are often undetectable [93]; however, in patients with late stage metastatic disease, levels of ctDNA have been shown to be significantly associated with progression free survival (PFS) and response to treatment [94, 95]. In a longitudinal assessment of ctDNA in patients treated with PD-1 inhibitors, a favorable ctDNA profile (undetectable ctDNA at baseline and during treatment) predicted OS, PFS, and tumor response to treatment compared to an unfavorable ctDNA profile (detectable ctDNA at baseline and during treatment) [96]. ctDNA may also be useful for monitoring development of resis-

tance to treatment, particularly targeted therapy, by detecting resistance mutations along with monitoring disease progression [94, 97].

16.2.2.6 DNA Methylation

Epigenetic changes of ctDNA, such as DNA methylation, are detectable in peripheral blood and are actively being investigated for their use as biomarkers in a number of cancers [98]. In melanoma, hypermethylation of a number of genes (RAR-beta2, RASSF1A, IDH1, CDKN2A) has been identified and shown to have prognostic and therapeutic significance [99, 100]. Hypermethylation of genes involved in tumor suppression and DNA repair such as RASSF1A, MGMT, and RAR-beta2 have been associated with poorer survival and treatment response [100–104]. A comprehensive DNA methylation analysis of all stages of melanoma revealed a prognostic signature of three genes (MEOX2, OLIG3, and PON3) for which the degree of DNA methylation may predict the prognosis of melanoma patients [105]. More recently, Guo et al. identified a prognostic four-DNA methylation signature independent of all clinical factors with high predictive performance for patients in early stages and with tumor thickness less than 2 mm [106]. In addition, DNA methylation profiles from melanoma tumors have been shown to be distinct from other tumors and methylation profiles of healthy controls [101].

16.3 Serologic Biomarkers

16.3.1 Lactate Dehydrogenase (LDH)

Lactate dehydrogenase (LDH) is essential for anaerobic glycolysis and is frequently upregulated in tumor cells, providing a survival advantage in a hypoxic environment. While LDH is not specific to melanoma progression, it is the strongest independent prognostic factor for melanoma progression in late stage disease [107]. Serum LDH is the only marker so far that has been incorporated into the AJCC melanoma staging and classification system [108] and is recommended as part of the standard workup following identification of metastatic disease by the 2020 NCCN guidelines [64]. In a meta-analysis of 7972 patients with stage IV melanoma, elevated serum LDH was an independent and significant predictor of survival outcome with 1- and 2-year OS rates of 65% and 40%, respectively, for those with normal serum LDH compared to 32% and 18% for those with elevated serum LDH [108]. Serum LDH is commonly used in the management of patients with late stage melanoma; however, due to its low specificity, false positive results are common from other conditions involving hemolysis, necrosis, and apoptosis, and it has not been helpful in distinguishing patients with early stage melanoma from healthy controls [109].

16.3.2 S100

Serum S100 Beta (S100B) is an indicator of tumor burden and has been correlated with tumor stage, survival, and recurrence [110, 111]. S100B has also been shown to be more specific for melanoma metastases compared to LDH [112]. In a meta-analysis of 3393 patients with stage I to IV melanoma, S100B positivity was associated with significantly poorer survival in all stages of melanoma [113]. However, other studies have failed to find any prognostic significance in patients with microscopic disease or those who are clinically tumor-free after surgery [114–116]. Egberts et al. found baseline serum levels of S100B to be significantly associated with treatment response in stage IV melanoma patients along with a strong correlation between treatment response and unchanged or declining S100B levels over time [109]. Higher S100B levels at baseline and increases over time are associated with poorer RFS and OS [117]. Increasing S100B levels during treatment may indicate that another treatment strategy is needed [117].

Although S100B is a more specific serum marker for melanoma than LDH, it may also be elevated in CNS, liver, renal, and cardiovascular disease [118, 119]. In clinical practice, S100 is primarily used only in European countries to monitor treatment response in advanced meta-

static melanoma given its relative unreliability for screening and detection in stage I and II disease [113].

16.3.3 Melanoma-Inhibiting Activity (MIA)

Although numerous serum biomarkers have been studied for their prognostic significance in melanoma, none have shown a higher sensitivity-specificity profile than LDH or S100B. Serum melanoma-inhibiting activity (MIA) is a protein highly expressed and secreted from melanoma cells. In a study of 112 patients with melanoma, 13% of patients with stage I disease, 23% with stage II, and 100% in stage III and IV were found to have elevated serum MIA levels. Furthermore, of 350 patients with a history of stage I/II melanoma who had been declared tumor free after surgical resection, 32 patients developed positive MIA values of which 15 had developed metastases, suggesting serum MIA may be useful to identify metastatic disease progression [120].

16.3.4 Circulating Melanoma Cells (CMCs)

In order to metastasize, tumor cells must leave the primary tumor site and intravasate into the bloodstream or lymphatics. The detection of circulating melanoma cells (CMCs) in the peripheral blood of melanoma patients has demonstrated prognostic value [121–125]. In a meta-analysis of 5433 patients, CMC status correlated with disease stage and OS [124]. In a retrospective analysis of 44 patients with melanoma, patients with two or more CMCs detected in peripheral blood were found to have an OS of 2.0 months versus 12.1 months for those with less than two CMCs detected [126]. The use of CMCs as a biomarker in the clinic is limited due to controversy surrounding the sensitivity, specificity, and reliability of CMCs as a biomarker given the high heterogeneity of CMCs along with differences in CMC collection and analysis [127]. To combat the heterogeneity of CMCs, Aya-Bonilla et al. used a multi-marker approach taking into account up to 19 genes. In these studies, CMC detection was associated with poorer OS and PFS while changes in plasma CMC concentration were found upon treatment initiation [128].

16.3.5 Exosomes

Exosomes are secreted cellular vesicles with a molecular profile characteristic of the cell of origin. Recent studies have identified unique mRNA, miRNA, and protein profiles in exosomes secreted by melanoma cells [129]. Lazar et al. identified a proteome signature present in exosomes from aggressive melanoma cell lines enriched in proteins involved in cell motility, immune response, and angiogenesis [130]. Analysis of exosomes from human melanoma tumors revealed a “melanoma signature” comprised of TYRP2, VLA-4, HSP70, and MET. Of patients with stage IV disease, those with protein-poor exosomes (<50 ug/mL) were found to have a survival advantage versus those with protein-rich exosomes (>50 ug/mL) [131]. Analysis of exosomal miRNAs from melanoma patients revealed significantly higher levels of miR-17, miR-19a, miR-21, miR-126, and miR-149 in patients with metastatic sporadic melanoma compared to familial melanoma patients and healthy controls [132].

16.4 Biomarkers of Treatment Response: Immunotherapy

The only biomarkers recognized by the 2020 NCCN guidelines with potential utility for immune therapy include programmed death-ligand 1 (PD-L1) expression and somatic mutation burden [64]. High PD-L1 expression (>5%) may be a marker for equivalent outcomes with nivolumab monotherapy compared to nivolumab and ipilimumab combination therapy in patients with metastatic melanoma [133]. Currently, PD-L1 is the only FDA-approved ICI biomarker which serves as a companion test for pembrolizumab treatment (PD-L1 IHC 22C3 pharmDx).

Tumor mutational load may also be predictive of response to ICIs. High mutational load in tumor tissue has been associated with OS in patients treated with CTLA-4 inhibitors and PD-1 inhibitors [134]. Further, exome analysis of tumor mutational load has revealed T-cell responses against patient-specific neoantigens [135]. A higher mutational burden may predict a more robust T-cell response. In a retrospective cohort of 173 patients with metastatic melanoma, Queirolo et al. identified two single nucleotide variants of the CTLA-4 gene that correlate with OS in those treated with anti-CTLA-4 therapy (3-year OS of ~30% versus ~13%), which may be used to predict patients with favorable outcomes to CTLA-4 therapy [136].

Many of the potential biomarkers being looked at for immune checkpoint inhibitor (ICI) response are involved in known immune response pathways. An effective response to ICIs is dependent on T-cell infiltration of the tumor microenvironment (TME) [137]. Early studies focused on serologic factors that may predict response to ICIs, including lymphocyte and eosinophil count, both of which are positively associated with improved survival [138–144]. In contrast, an elevated neutrophil count or high neutrophil/lymphocyte ratio (NLR) in patients treated with monotherapy ipilimumab (an anti-CTLA-4 antibody) or nivolumab (an anti-PD-1 antibody) was associated with poor OS or no response [141, 144–147].

Other serologic biomarkers such as LDH and C-reactive protein (CRP) have also been looked at in the context of immunotherapy. Elevated LDH and CRP at baseline and during treatment have been found to be significantly associated with poorer OS in patients treated with ICIs [141–143, 148]. Other proposed serum biomarkers include IL-8 and angiopoietin-2. IL-8, which may be secreted by melanoma tumor cells, has been found to be inversely correlated with OS in melanoma and non-small cell lung cancer (NSCLC) patients treated with PD-1 inhibitors [149]. High baseline and increasing angiopoietin-2 levels during treatment have been associated with reduced OS in PD-1 and CTLA-4 inhibitor-treated patients [150].

Cellular biomarkers are also being investigated to predict treatment response to ICIs. Subrahmanyam et al. found subsets of CD4+ and CD8+ T cells to vary between responders and non-responders to anti-CTLA-4 treatment, while subsets of natural killer (NK) cells were shown to correlate with clinical response to anti-PD-1 therapy [151]. Others have observed an increased response to PD-1 inhibitors in patients with greater tumoral CD8+ T-cell infiltration and PD-1/PD-L1 expression pre-treatment [152, 153]. In patients treated with anti-PD-1 therapy, the presence of PD-1+ CTLA-4+ cells within the tumor-infiltrating CD8+ T-cell population was found to significantly correlate with response to therapy and PFS, which was 31.6 months in those with tumors with more than 20% PD-1+ CTLA-4+ CD8+ T cells compared to 9.6 months for tumors with 20% or fewer [154].

Another marker of response to treatment with ICIs may be immune-related adverse events (irAEs) during treatment. Downey et al. observed increased efficacy of anti-CTLA-4 treatment in patients who experienced irAEs (26% objective responders) compared to those who did not (2% objective responders). The severity of irAE seemed to correlate with response as those with high grade irAEs (grade 3–4) showed an even greater objective response [155]. Blank et al. proposed using an "immunogram" looking at seven different parameters (mutational load, T-cell infiltration, expression of immune checkpoints, CRP/IL-6, lymphocyte count, and expression of MHC class I) to predict response to immunotherapy. This builds on the observation that (1) the outcome of cancer-immune interactions depends on many unrelated parameters such as T-cell inhibitory mechanisms and tumor "foreignness" and (2) the value of the parameters may vary significantly among patients [156].

16.5 Biomarkers of Treatment Response: Targeted Therapy

Screening for BRAF and NRAS mutations is currently routine in the management of cutaneous melanoma while KIT mutations are evaluated in

melanomas in sites of chronic sun exposure, acral sites, and mucosal melanomas. According to the 2020 NCCN guidelines, BRAF mutation testing and, in the appropriate clinical setting, KIT mutation testing is recommended upon initial presentation with stage III or IV disease or clinical recurrence [64]. Identification of a BRAF or KIT mutation/amplification in melanoma allows for the use of effective targeted therapies in patients harboring these tumors. Treatment of patients with tumors harboring V600E BRAF mutations with BRAF inhibitor (BRAFi) monotherapy or combined MEK inhibition (MEKi) has demonstrated complete or partial tumor regression in the majority of patients [157, 158]. On the other hand, BRAFi use is not recommended and available evidence suggests there is no benefit in treating patients without V600E BRAF mutations [64, 159]. KIT mutations are observed to occur in "hotspots" across the gene and demonstrate variable sensitivity to KIT inhibitors with observed disease control rates around 50% in patients with KIT mutations [47–49]. NRAS-mutant melanomas are generally unresponsive to targeted therapies and are therefore generally treated with ICIs in advanced disease.

BRAF-mutant ctDNA has been widely studied, and high baseline levels have been found to be associated with poor response to MAPK-inhibitor (MAPKi) therapy, alone or combination [160–163]. In a prospective analysis of 48 patients with advanced metastatic melanoma treated with targeted or immunotherapy, lower BRAF-mutant ctDNA levels pre-treatment were significantly associated with response to treatment and longer PFS, regardless of treatment type. However, levels of ctDNA decreased significantly corresponding to response to therapy in those treated with targeted therapy, unlike those receiving immunotherapy [94].

In a retrospective analysis of 617 patients with BRAF-mutant melanoma treated with dabrafenib plus trametinib; LDH level and number of metastatic disease sites (less than three) were significantly associated with PFS and OS [164]. Wang et al. identified cancer-specific extracellular vesicle (EV) phenotypes in melanoma patient plasma and identified specific EV profiles associated with resistance to targeted therapy [165]. Recently, an analysis of 90 patients with BRAF V600-mutant melanoma treated with either BRAFi alone or combined with a MEKi revealed PFS of 9.1 and 3.5 months, respectively, and OS of 17.2 and 5.5 months, respectively, for patients with NLR less than 5 and NLR greater than or equal to 5 [166].

Recent studies have explored gene signatures and genetic profiles associated with response to targeted therapy. In a retrospective study of 64 patient tumor samples treated with BRAFi monotherapy, pre-treatment overexpression of a subset of genes was significantly associated with PFS and OS [167]. In a retrospective analysis including patients with BRAF V600-mutant metastatic melanoma treated with vemurafenib with or without cobimetinib from BRIM-2, BRIM-3, BRIM-7, and coBRIM studies, whole exome sequencing revealed alterations in MITF and TP53 were more frequent in tumors from patients with rapid progression, while alterations in NF1 were more common in tumors from patients with complete response. In addition, RNA sequencing analysis revealed enrichment of genes associated with immune response in those patients with complete response, while genes related to keratinization were enriched in tumors from patients who experienced rapid progression [168]. Wongchenko et al. identified two gene signatures, immune and cell cycle, from patients in BRIM-2 and BRIM-3, of which, the cell-cycle gene signature was associated with shorter PFS in those treated with vemurafenib monotherapy [169]. Others have noticed a higher baseline PTEN expression to be associated with response to vemurafenib monotherapy [170]. Wagle et al. constructed a MAPK pathway activity score focusing on the expression of 10 MAPK target genes and found a higher score to be associated with improved PFS [171].

16.6 Summary

While investigators have been evaluating the potential utility of diagnostic and prognostic melanoma biomarkers for decades, more recent advances in the development of effective melanoma therapies targeting driver mutations in mel-

anoma have informed the development of biomarkers predictive of treatment effectiveness and the monitoring of treatment responses. Emerging molecular technologies are currently being developed to provide meaningful diagnostic and prognostic information for melanoma; however, insufficient data currently exists to make such technologies clinically useful. As additional data accumulate regarding resistance mechanisms to targeted therapies and immunotherapies, we expect new biomarkers will be developed to detect early treatment resistance in patients and support therapies to overcome treatment-specific resistance mechanisms in melanoma (Table 16.1).

Table 16.1 Biomarkers used for prognosis and treatment response in melanoma

Biomarker	Study Cohort	Correlation	Methodology	References
Molecular biomarkers				
Ki-67	688 patients with primary melanomas 202 patients with nodular melanoma 68 patients with melanoma ≥4 mm thick	PFS, OS OS PFS, OS	IHC IHC IHC	Ostmeier et al. 2001 [26] Ladstein et al. 2010 [30] Robinson et al. 2018 [31]
MCAM	76 patients with stage IA to III 78 patients with primary melanoma, 92 patients with metastatic melanoma	OS OS, nodal progression	IHC IHC	Pacifico et al. 2004 [35] Pearl et al. 2007 [36]
Genetic biomarkers				
CDKN2A/B	74 relapsed patients, 42 nonrelapsed patients 112 melanoma tumor samples from 86 patients	Tumor thickness, mitotic rate, ulceration, risk of relapse OS	MLPA, PCR, IHC PCR, RTPCR	Conway et al. 2010 [59] Grafstrom et al. 2005 [62]
ctDNA	48 patients stage IV 92 patients stage IV, BRAF-mutant	PFS, treatment response PFS, treatment response	ddPCR RTPCR	Gray et al. 2015 [94] Ascierto et al. 2013 [95]
Serologic biomarkers				
LDH	30,946 patients stage I-III and 7972 patients stage IV 50 patients stage I-II, 61 patients stage IV	OS Tumor stage	Meta-analysis Photometric assay	Balch et al. 2009 [108] Egberts et al. 2011 [109]
S100B	3393 patients stage I-IV 50 patients stage I-II, 61 patients stage IV. 20 patients stage III-IV 670 patients stage IV	OS Tumor stage, survival, treatment response Metastasis (75% sensitive, 92% specific) OS, RFS	Meta-analysis Photometric assay ELISA Chemiluminescence	Mocellin et al. 2008 [113] Egberts et al. 2011 [109] Oberholzer et al. 2008 [110] Tarhini et al. 2009 [117]
MIA	112 patients stage I-IV 350 patients stage I-II	Prognosis Metastasis, disease progression	ELISA	Bosserhoff et al. 1997 [120]
CMCs	5433 patients stage I-IV 44 patients stage III-IV 43 patients stage IV	Disease stage, OS, PFS OS OS, PFS	Meta-analysis Automated CTC assay IHC, RTPCR, ddPCR	Mocellin et al. 2006 [124] Rao et al. 2011 [126] Aya-Bonilla et al. 2020 [128]

PFS Progression free survival, *OS* Overall survival, *IHC* Immunohistochemistry, *MLPA* Multiplexed ligation-dependent probe amplification, *PCR* Polymerase chain reaction, *RTPCR* Real-time PCR, *ddPCR* Droplet digital PCR, *RFS* Relapse free survival, *CMCs* Circulating melanoma cells, *CTC* Circulating tumor cells

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